FOREWORD

Beyond the Historical Perspective on Hydrogen and Electron Transfers

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Abstract

A brief overview of proton and electron transfer history is given, and various features influencing enzymatic catalysis are discussed. Examples of generic behavior are considered, together with questions that can be addressed for both experimental and computational results. Examples of high and low pre-exponential factors A of the intrinsic rate constant k_H ranging from $\sim 10^{17}\,s^{-1}$ to $\sim 10^{4}\,s^{-1}$ and normal ($\sim 10^{13}$) are noted with significant error bars and discussed.

This series of chapters covers almost every aspect of reactions in enzyme catalysis from many leading participants in the field. They range from pedagogic descriptions of the relevant quantum theory and quantum/classical theoretical methodology to the description of experimental results. The theoretical interpretation of these large systems includes both quantum-mechanical and statistical-mechanical computations, as well as simple more approximate models.

Most of the chapters focus on enzymatic catalysis of hydride, proton and H$^{\cdot}$ transfer, an example of the latter being proton-coupled electron transfer. There is also a chapter on electron transfer in proteins, timely since the theoretical framework evolved some fifty years ago for treating electron transfers has been adapted to H-transfers and electron transfers in proteins. It is perhaps therefore of some interest to recall briefly some of the early history in the proton- and electron-transfer fields, briefly since the history covers some 85 or so years.

Brönsted's treatment of acid–base catalysis originated in the 1920s and involved in part the transfer of a proton from one reactant to another. It focused on linear kinetic-thermodynamic plots such as the logarithm of the reaction rate *vs*. some thermodynamic measure of the effect of the driving force of the reaction, for example, the logarithm of an acid or base strength (dissociation constant). These linear free-energy plots were subsequently applied to many other types of reaction rates in solution. A deviation from linearity was found by Eigen in the 1950s in his studies of very fast proton-transfer reactions. The deviation occurred at a high driving force. Ultimately, the reaction rate was limited by the rate of diffusion of the reactants toward each other. Many conferences were held on the theme of linear free-energy relations in chemical reaction rates.

In the late 1940s and in the 1950s, experiments on electron-transfer reactions between ions in solution differing only in their valence state were initiated using isotopes as radioactive tracers. These reactions form the simplest class of reactions in all of chemistry, no chemical bonds being broken or formed in some cases and there being zero chemical "driving force" – zero standard free energy of reaction. Such experimental studies provided information thereby on other factors that influence the reaction rate. Based on the results of such studies, Bill Libby in 1952, citing a suggestion of James Franck, introduced the notion of the Franck–Condon (FC) principle controlling the rate of electron transfer. Stimulated by Libby's work, I formulated in 1956 an electron-transfer theory. The task was to satisfy the FC principle without violating (as had previously been done) the law of conservation of energy during the electron transfer. A "reorganisation" of the system had to occur prior to and following the electron transfer in order to satisfy both criteria.

For simplicity, the solvent was treated as a dielectric continuum and a nonequilibrium dielectric polarisation of the solvent at every point was determined by converting the problem to one of thermodynamics of a system with a nonequilibrium dielectric polarisation in the transition state. One distinguished here between the fast (electronic) and slow (nuclear) polarisation of the solvent. In 1960 this work was extended using statistical mechanics instead of the dielectric continuum theory, and now included changes in nuclear configurations of the reactants (*e.g*., bond lengths and angles). To this end a global reaction coordinate was needed and was introduced to treat the system of some 10^{23} coordinates. The coordinate used was the energy of the products/solvent in their nuclear environment minus that of the reactants/solvent with the same values of the nuclear solvent/vibrational coordinates (the vertical energy difference of the two 10^{23} or so dimensional potential-energy surfaces). It was possible in this way to reduce the description to that of a one-coordinate plot of the free energy of the reactants/solvent (a parabola) and that of the products/solvent along the reaction coordinate (a parabola) and calculate the free energy of activation, the transition state occurring at the intersection of the two parabolas.

The outcome of the theory were many predictions of relations between various types of rate constants, including the effect of driving force, and a hitherto unsuspected effect termed in this 1960 paper the "inverted effect." It

was verified indirectly some years later, but required some 25 years before a direct verification was made. Much of the brief electron-transfer history mentioned here and the vitally important underlying experimental work has been described in more detail in my Nobel Lecture, as well as in many articles and books by other contributors.

In the case of the transfer of H^+, H^- or $H^\cdot$, we again have a transfer of a light particle. The Franck–Condon principle applies, though more weakly, when the mechanism is that of ''jumping'' of the H from one reactant to the other, a so-called nonadiabatic H transfer. For purposes of understanding some computations and formulating an approximate theory a ''protein reorganisation'' prior to the H-transfer is treated in this approach, the system proceeding from configurations of the atomic nuclei in the system favourable to the reactants to those favourable to the products *via* those favourable to the transition state (TS). The concepts and the mathematical formalism have been adapted to H-transfers in several ways and are described in a number of chapters in this book.

When the H transfer is not as sudden, for example, in an ''adiabatic H transfer'', the reaction $AH + B \rightarrow A + HB$ (charges not shown) has strong electronic coupling between A and H and between H and B in the transition state. Some deviation from the simple nonadiabatic picture is expected and has been treated in several ways and in some analytic approximations given in the present volume. To treat this case, a different reaction coordinate has also been introduced into many of the computations, such as the length of the incipient newly forming bond minus that of the rupturing bond, for each nuclear configuration of the entire system. (In the enzyme the new coordinate goes from some small negative value of the order of one Å to a positive value of the same order.) We discussed (2007) the challenges of combining in approximate models the ''nonadiabatic'' nonequilibrium polarisation of the reorganisation of the protein with the adiabatically behaving AHB. The use of computational methods is permitting the detailed investigation of various aspects of adiabatic/nonadiabatic H transfer.

One principal focus in this book, and indeed in its title, is H-tunnelling, introduced into the chemical reaction rate literature in the 1930s by R. P. Bell. He assumed fixed positions of the heavy nuclei. Now, some seventy years later, more advanced treatments are used, as seen in the present volume. For enzymes the effect of tunnelling on the kinetic isotope effect (KIE, k_H/k_D) is as large as a factor of the order of 100 or as small as a factor of the order of unity, depending on the system and the experimental conditions. Tunnelling can also occur for reactions in solution of course. In either case tunnelling can be a large effect, though usually smaller than a factor of the order of 100. (The KIE itself is usually smaller than a factor of 100, and part of that is often due to zero-point energy differences for the H and D systems.) It is small compared with a catalytic effect of many orders of magnitude for reactions in enzymes relative to the rates of the corresponding reactions in solution. Nevertheless, the KIE and its temperature dependence are highly instructive.

Two types of $H^\cdot$ transfer have been described in the literature and are found in this volume, a hydrogen-atom transfer (HAT) in which the proton and

electron are transferred from the same atom and another class of proton-coupled electron transfers (PCET) in which they are transferred from different atoms. Examples of both types are considered in this volume.

To understand better the enzymatic results it has been necessary to disentangle the overall reaction rate from the contributions due to the binding of the substrate reactant and of the substrate product to the enzyme – the "commitments". The rate constant corrected for these commitments, *i.e.* the rate corrected to 100% binding of the substrate reactant and the product (the "intrinsic rate"), is of particular interest in theoretical analyses of the actual H-transfer step. In the study of the intrinsic rate, both the pre-exponential factor A and the activation energy provide added insight. We cite later some examples of A.

In the precomputer age of chemistry the emphasis in theory was on equations, Brönsted, Debye–Hückel, Onsager, Kramers, transition-state theory, RRKM, and many others, and particularly on the functional form of the equations and their applications to interpret and predict experimental data. In fortunate cases, as in electron-transfer reactions, one can relate different properties using the equations without adjustable parameters. Now, with the advent of modern computers, there is an emphasis on the final results of the theoretical computations for individual systems. Indeed, one can now get detailed answers to questions for individual systems that one couldn't obtain in earlier days, the accuracy of the answers depending on the validity of the approximations made in the model. This volume contains examples of these valuable analyses. One of the few aspects not covered, the use of nuclear magnetic resonance techniques to study couplings and motions in proteins, has been the subject of several recent reviews in the literature.

One might ask what relationships or generalities are there in these enzyme systems, with qualitative or approximately quantitative answers. As we noted in an article in 2007 some of the relationships between experimental data that were predicted for electron transfers are addressable for H-transfers in solution but not for H-transfers in enzymes. For example, one does not have the crossrelations in enzymes relating the rate constants of crossreactions to those of the component self-exchange reactions, nor does one have the wide range of driving force available to proton and electron transfer in solution. In addition in the field of electron transfers one has the relation of the kinetic properties to charge-transfer spectra* and to the rates of ET reactions at electrodes. These predicted relationships and comparisons with experimental data enriched and enlarged the field. The electron-transfer formalism that has frequently been adapted to H-transfers in solution and in proteins includes several features:

(1) There is a work term w_r, a preorganisation of the reactants prior to any chemical change in the bonding and similarly a work term w_p for the reverse reaction, a preorganisation of the products. (The standard free energy of the H transfer step ΔG° was partitioned thereby into a sum $\Delta G^\circ_R + w_r - w_p$). For the

* A rare example of a charge-transfer absorption for a group transfer was described for the $I^- \bullet CH_3I$ system by D.M. Cyr, C.G. Bailey, D. Serxner, M.G. Scarton, M.A. Johnson, *J. Chem. Phys.* 1994, **101,** 10507.

electron transfers for two approaching ions in solution w_r consisted of the electrostatic repulsion of the ions (and any other free-energy barrier that could not be reduced by favourable chemical alteration of the ΔG° for the actual transfer step). Extended to enzymes, w_r now includes both the free energy "barrier" due to a prior selection of substrate/cofactor separation distances, orientations and protein conformations before any protein and bond length/angle "reorganisation". One cannot obtain w_r experimentally simply by finding the free-energy barrier that exists that cannot be overcome by a sufficiently favourable ΔG°_R, since a large variation of the latter is not practical for enzymes, in contrast to transfers in solution. So there will remain a judgment call on what part of the overall ΔG° to include in w_r. The w_r can be entropic or energetic in nature. For a wild-type enzyme operating at its natural temperature and with its natural substrate and coenzyme this w_r is expected to be smaller than its value for other experimental conditions and than for some mutants. The distance sampling in w_r is sometimes called gating, but the latter can also include additional contributions, such as reorientation of a blocking group.

(2) There is a "reorganisation" of the protein, as well as a change of distances within the reactants (more generally of their geometry) so as to facilitate the H-transfer. The protein reorganisation is approximately a harmonic function of the relevant coordinate, a parabola. The role of bond breaking-bond forming in AHB has been taken into account in several alternate ways. In one approach (empirical valence bond) it is treated *via* a pair of free-energy profiles that are approximately a pair of parabolas for the free energy of reactants and of products, and the resulting energy barrier is corrected by lowering it by a term that contains the element H_{AB} coupling the two valence states, (AH,B) (A,HB). In another approach (2007), the protein reorganisation is again treated using a pair of parabolas for the formation of the TS but a bond-breaking/bond-forming formalism is used for the AHB subsystem. A challenge in the 2007 paper was to combine these quite different approaches for the protein and AHB and calculate the free energy of formation of the TS. The result deviates somewhat from harmonic behaviour in its dependence on ΔG°.

(3) Introduction of tunnelling and the over the barrier crossing contributions of the H-transfer step completes the expression for the rate. The tunnelling depends on the separation distance R as do w_r and the protein reorganisation. The tunnelling contribution in this approximate analysis is calculated at each R and involves an average over R using this R-dependent expression as a weighting factor. The equation for the rate constant of the reverse reaction k_H^{rev} is obtained similarly, and the theoretical expression can be tested to see if the resulting equilibrium constant k_H/k_H^{rev} is independent of w_r and w_p, as it should be.

While detailed computations have been and continue to be highly instructive, it is also useful to consider, as in any field, whether any generalisations, actual or potential, have emerged for enzyme catalysis from the experiments or from the computations or both. Several possibilities are noted below, phrased in part as questions. Their validity and that of others can be explored in further experiments.

(1) Is the intrinsic KIE, namely the ratio of the intrinsic rate constants for H-transfer and D-transfer, k_H/k_D, largely temperature independent for wild-type

enzymes operating with their natural substrates in their natural temperature range of operation, *e.g.*, as in some recent results?[1–9] One might have argued that such a temperature independence is the result of two opposing tendencies, and that they tend to cancel for these systems. The case of a lipoxygenase,[9] which shows a weak temperature dependence discussed in this volume, may differ from some other enzymes[1–8] whose KIE shows essentially no temperature dependence (above any "breakpoint" discussed in (2) below). If this temperature independent behaviour for the intrinsic KIE proves to be widespread, then a delicate balance between two opposing trends appears unlikely. A necessary condition can be imagined for k_H/k_D to be temperature-independent, namely that there be little or no w_r arising from the stretching of the rupturing H-bond (hence, no isotopically sensitive contribution). A protein reorganisation barrier exists but cancels in the ratio k_H/k_D, and both H and D are transferring from their lowest vibrational state.

(2) Is there some generic explanation as to why a "breakpoint" occurs for some enzymes? At temperatures above a breakpoint the KIE is temperature independent and at temperatures below the breakpoint the KIE is temperature dependent.[1,5,8] In the case of a particular dihydrofolate reductase[5] the k_D showed a breakpoint but not k_H, while for a thermophilic alcohol dehydrogenase[1] both plots showed a breakpoint. In each case, the KIE, k_H/k_D, showed a breakpoint. Essentially it is like a phase transition, with a sharp change of the properties of k_H/k_D. Both the activation energy and the entropy of activation for k_H/k_D changed. I don't recall that this rather abrupt change has been captured as yet in computations. Models can be suggested and explored to understand these results. There can be artifacts if the rate constant is not corrected for the commitments. For example, the observed KIE for a dihydrofolate reductase showed a breakpoint but the intrinsic KIE did not.[7] At lower temperatures, the commitments presumably became more important and, being isotopically insensitive, reduced the KIE towards unity.

(3) Is the pre-exponential factor A in the intrinsic rate constant, $k = A\exp(-E_a/kT)$, smaller than the typical value $10^{13}\,s^{-1}$ for a certain class of reactions? For example, data for soybean lipoxygenase, believed to be a proton-coupled electron-transfer reaction, show that at 30°C $k_H \sim 300\,s^{-1}$ and $E_a \sim 2$ kcal mol^{-1}.[9] Thereby, $A \sim 10^4\,s^{-1}$ and so is far smaller rather than $10^{13}\,s^{-1}$. The small A is attributed to poor overlap of the relevant vibrational and electronic wavefunctions. Again, can A be much larger than $10^{13}\,s^{-1}$ at temperatures below a breakpoint for some protein? A thermophilic alcohol dehydrogenase has a breakpoint around 30 °C.[1,10,11] The rate constant k_H there is $25\,s^{-1}$. For $T < 30$ °C we have $E_a \sim 21$ kcal mol^{-1} and so $A \sim 10^{17}\,s^{-1}$, while just above 30 °C we have $E_a \sim 14.5$ kcal mol and a normal value of A, $A \sim 10^{12}\,s^{-1}$.[10,11] The high A for $T < 30$ °C cannot be explained by sampling a small subset of reactive conformations. The sampling would create an A much less than $10^{13}\,s^{-1}$ rather than much greater. We note that apart from tunnelling we have $A \sim 10^{13}\exp(\Delta S^{\dagger}/k)\,s^{-1}$, where $\Delta S^{\dagger}$ is the entropy of activation of the H-transfer step. The difference in protein flexibility in the initial state above and below the breakpoint has been discussed,[10,11] using results on the rate of hydrogen-deuterium exchange. A breakpoint has been observed in two thermophilic

enzymes[1,5] and in a mutant thereof. We return later in some concluding remarks to an analogous behaviour on a very high A that we have noticed for viscous systems and so to a possible explanation of a very high A below the breakpoint.

There is another factor that influences A, and can be expected to arise in some cases (it has not been tested for enzymes, though it is known for reactions in solution). If the standard entropy of reaction ΔS° for that step is different from zero, then when $|\Delta G^\circ|$ is small $\Delta S^\dagger$ contains a term $\sim \Delta S^\circ/2$ arising from this contribution, using the two-parabola formalism for the protein reorganisation. When the reaction is a charge-shift reaction, such as $AH^- + B \rightarrow A + HB^-$, then ΔS° may be close to zero. However, in the case of a charge separation ΔS° can be quite negative, while for charge recombinations it can be quite positive, due to the effect of the charges on the polarisation of the dipolar groups in the protein surrounding AHB. In a model reaction for lipoxygenase ΔS° has been measured for a hydrogen-atom transfer (HAT) where a dicatonic Fe (III) complex is formed from a Fe (II) complex,[12] as well as for other central metal atoms.[13] The ΔS° was very negative, ~ -30 e.u., reflecting the extra stiffness (more polarised) state of the more highly charge-separated product, contributing *via* $\sim \Delta S^\circ/2$ a factor of $\sim 10^{-3}$ to the pre-exponential factor A.

(4) In some cases mutations some 20 Å from the active site can have a dramatic effect on the catalytic rate.[14] How general is this phenomenon and does its origin lie in an effect on the reorganisation term λ *via* some hydrogenic bonded network, or some effect *via* the network on the local steric properties of the substrate–coenzyme pair and hence on w_r? The possible effect of mutants on the network is discussed in this volume.

(5) In a comparison of two intrinsic KIEs, k_H/k_D, and the carbon isotope effect, $^{12}k/^{13}k$, do they show the same trend as a function of pressure, at least at higher pressures, as in a unique study in the literature[15] (in particular they have a similar value of the intrinsic volume of activation) perhaps reflecting that the C atom is a component of the reaction coordinate in the TS. (Only in a special case is the H-tunnelling a purely H motion.) The effect of pressure on the H/D KIE, k_H/k_D, and on k_H and k_D was studied recently using a single-turnover stopped-flow apparatus.[16] Since the reacting substrate–cofactor complex was fully bound, no correction for commitments was needed. The ratio k_H/k_D increased with increasing pressure, as did k_H and k_D. Previously steady-state experiments had been made for different enzymes, and the current situation on steady-state and stopped-flow measurements is described in a chapter in the present volume.

The above discussion on the theoretical aspects focused on TS theory, a statistical reaction-rate theory, and one might ask what dynamical aspects can one consider to interpret the behaviour of the protein below the breakpoint. For example, if the protein at temperatures below the breakpoint is sufficiently rigid that the ''internal viscosity'' of the motion along the reaction coordinate becomes rate controlling, this motion would be diffusive in nature, leading to many (diffusive) recrossings of the transition state region and so, using the arguments introduced by Wigner[17] in his seminal 1938 paper, lead to a rate lower than that given by transition-state theory. Recrossings of the region of

the TS for enzymatic reactions are frequently calculated in current computations, as in this volume. Usually the factor is close to unity, but the value for a "viscous phase" of the protein may not have been studied. To consider the behaviour below the breakpoint it is useful to summarise some earlier studies on recrossings.

We first recall that the role of recrossings in reducing the reaction rate below the TS value can been seen both in Wigner's important explanation[17] (recrossings cause some of the phase space of the TS to be "wasted") and explicitly in the equations of Kramers[18] in his celebrated 1940 theory on an internal frictional effect on reaction rate. This characterisation as celebrated is perhaps not inappropriate since his article has received more than 4000 citations.

The classical results in Kramers' article are for a simple one-coordinate model, but they can be extended approximately to a multidimensional system by introducing a free-energy curve instead of the original one-dimensional potential-energy curve. His TS expression for the rate constant is then $k = \nu \exp(-\Delta G^*/kT)$, where ΔG^* is the free-energy barrier to reaching the TS (in the present case the sum of w_r plus the contributions due to the protein reorganisation and due to the bond breaking-bond forming terms). The ν is the frequency for motion along the reaction coordinate at the bottom of the reactants' free energy well. At the other limit, the limit of high internal friction coefficient ζ along the reaction coordinate, we have the Kramers' equation for the "overdamped" limit, $k = (2\pi\nu\nu'/\zeta)\exp(-\Delta G^*/kT')$, when $\zeta/2 \gg 2\pi\nu'$. Here, ν' is the frequency of the inverted parabola at the top of the TS barrier. With ν and $\nu' \sim 10^{13}\,s^{-1}$ and $\zeta/2 \gg 2\pi\nu'$ in the overdamped regime, this $2\pi\nu\nu'/\zeta$ factor in the rate constant k is less than $10^{13}\,s^{-1}$, in agreement with an interpretation in terms of recrossings of the TS in this regime.

Some insight into the very high pre-exponential factor of the rate constant at temperatures below the breakpoint can be obtained by comparing the pre-exponential factors in viscous *vs.* nonviscous media obtained from the Kramers' expressions for the rate constant k. A value of ζ can be estimated from a diffusion constant D and the Einstein equation, $\zeta = kT/mD$, or from the viscosity η and the Stokes equation, $\zeta = 6\pi\eta r/m$, where m and r denote the molecular mass and radius in the liquid (or $\zeta = 4\pi\eta r/m$ for a "stick" boundary condition). For a nonviscous liquid like acetonitrile ζ is about $10^{12}\,s^{-1}$, and so the reacting system is not "overdamped". For a viscous liquid like glycerol at 30 °C ζ is about $10^{15}\,s^{-1}$, so $2\pi\nu\nu'/\zeta$ is significantly less than ν and the system is overdamped. The pre-exponential factor in $2\pi\nu\nu'/\zeta$ for glycerol is about 10^{20} to $10^{23}\,s^{-1}$ depending on the temperature regime. Similarly, for a system such as silica above and below a transition temperature T_g one can calculate from the data[19] that the pre-exponential factor for the viscosity is $\sim 10^6$ times smaller at temperatures below T_g. When introduced into the Kramers' expression for the (overdamped) rate constant this factor yields a pre-exponential factor greater by a factor of $\sim 10^6$ than that at $T > T_g$. The motion for $T < T_g$ has been treated in the literature as hopping between structures, and as more fluid like for $T > T_g$.

Kramers' work has been extended to include a frequency-dependent friction $\zeta(\nu')$ (Grote-Hynes) and to treat reactions where the reaction coordinate has a

fast component plus Kramers' slow diffusive component (Agmon and Hopfield; Sumi, Nadler and the author; Hynes). In both modifications the rate constant will depend less on the low-frequency ζ used above, but nevertheless the high A value found below the breakpoint may have the same origin as its counterpart in other viscous systems.

Current computations do not treat as yet the dynamics of the slow timescales of milliseconds for the overall H-transfer. Nevertheless, they may eventually be able to treat the dynamics of the short individual diffusive steps below the breakpoint at various points along a reaction coordinate and so provide further insight into the properties of the protein below the breakpoint.

The field of enzyme catalysis and H-tunnelling has seen an explosion of studies and understanding. This book provides a volume rich in its breadth and depth for experienced researchers in the field and for those new to it. It is a field where questions and challenges abound.

Bibliography and Acknowledgements

Many authors have made major contributions to the electron-transfer and H-transfer fields, as seen in the copious citations in the present volume. I am indebted to Judith Klinman, Amnon Kohen, Sharon Hammes-Schiffer and Maria-Elisabeth Michel-Beyerle for their helpful comments. The research described in this preface was supported by the National Science Foundation and the Office of Naval Research. Recent reviews and other articles are cited in the author's *J. Phys. Chem. B,* 2007, **111**, 6643 and *J. Chem. Phys*., 2006, **25**, Art. No. 194504, which also elaborate on several of the topics mentioned here.

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